

LABORATORY EXPERIMENTS OF THE TRAIL FOLLOWING  
OF ARMY ANTS OF THE GENUS NEIVAMYRMEX  
(FORMICIDAE: DORYLINAE)

by

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## INTRODUCTION

The experiments performed and discussed in this thesis were designed to investigate three aspects of the trail following behavior of army ants: (1) Will one species of Neivamyrmex follow the trails of different species of army ants or other ants? (2) Will Neivamyrmex spp. follow trails made with alcohol extracts of other species? (3) What is the source of the Neivamyrmex trail material?

Neivamyrmex, formerly a subgenus of Eciton, was raised to a genus by Borgmeier (1953). Army ants of the genus Neivamyrmex are of Neotropical origin, and most of the 17 species occurring in the United States are found in the region from Texas westward through New Mexico and Arizona into the southern half of California. The exact distributions of the various species have not been accurately determined. Five species of army ants have been found in Kansas: Neivamyrmex nigrescens (Cresson), N. opacithorax (Emery), N. carolinensis (Emery), N. fallax Borgmeier, and N. minor (Cresson). Only males of N. minor and workers of N. fallax have been collected, but all castes of the other three species have been collected in the United States (Muesebeck, et al., 1951:780). Colonies of carolinensis, opacithorax, and nigrescens were studied during the summer of 1961 near Manhattan, Kansas. As far as is known, these ants are carnivorous, their food consisting largely if not entirely of insects (Smith, 1942:539; Rettenmeyer, 1962). Although some species are subterranean, others such as opacithorax, pilosus, and nigrescens, are not

strictly so. Workers of these species are commonly observed in columns on the ground, even in full sunlight (Smith, 1942:539). Temporary nests or bivouacs are usually found in rotten logs and stumps or in the soil beneath objects lying on the ground. All of the bivouacs or clusters which were found during the summer of 1961 were under rocks on grassy slopes. A colony was normally divided into several clusters of workers which occupied the space under several rocks in the same area. These clusters were usually connected by columns of workers moving in both directions. Colonies of Neivamyrmex usually have only one queen and approximately 150,000 to 250,000 workers (Schneirla, 1958:217).

Since army ants are almost blind, they are completely dependent upon their ability to lay and follow chemical trails during their raiding and periodic emigrations. Raiding columns of nigrescens and opacithorax range from 30 to 70 meters long; and emigration columns range from 6 to 64 meters long (Schneirla, 1958:241, 245). Although workers normally move in a continuous column from the nest, any member of a colony can follow any part of an established trail without previous experience on that particular trail and without the presence of other workers (Schneirla, 1944:2).

Almost all previous work on the trail following of army ants has been conducted in the field. The lack of controlled laboratory experiments has been due largely to the difficulties encountered in maintaining colonies of army ants in the laboratory.



# REVIEW OF LITERATURE

In referring to the behavior of Neivamyrmex carolinensis, Forel (1899:444, as translated by Wheeler [1910:2647]) wrote:

Throw a handful of army ants with their larvae on a spot with which they are absolutely unacquainted. In such circumstances, other ants scatter about in disorder and require an hour or more (sometimes less) to assemble and bring their brood together, and especially to become acquainted with their environment, but the army ants do this at once. In five minutes they have formed distinct files which no longer disintegrate. They carry their larvae and pupae, marching in a straight path, palpating the ground with their antennae and exploring all the holes and crevices until they find a suitable retreat and enter it with surprising order and promptitude. The workers follow one another as if at word of command, and in a very short time, all are in safety.

Wheeler (1910:265) reported that workers of nigrescens in the laboratory followed a never ending trail around the base of a glass jar for 46 continuous hours. He stated,

For nearly two whole days, these blind creatures, so dependent on the contact-odor sense of their antennae, kept palpating their uniformly smooth, odoriferous trail and the advancing bodies of the ants immediately preceding them, without perceiving that they were making no progress, but only wasting their energies.

Schneirla (1944) reported a case of circular milling of Labidus praedator which he observed at Barro Colorado Island, Canal Zone. Several hundred workers followed a circular trail in the open until they died of desiccation after 24 hours. He used this example to illustrate the psychological limitations of army ants in their stereotyped trail-following behavior.

Schneirla (1933:269, 273) said that the importance of the individual army ant is small; trail-formation is an activity that

involves a mass of behavior units. At the beginning of a raid, the ants pass out from the bivouac in small groups which he calls "pushing parties." The membership of these advancing bands constantly changes due to the fact that ants proceed hesitantly a few centimeters beyond previously "mastered" ground and then return toward the bivouac as newcomers push on in their turn. He states that the direction of formation of a trail and the fate of a trail depends upon the physical characteristics of the terrain and upon the supply of the booty in the territory tapped by the trail. Schneirla (1956) reported that removing a leaf or other surface over which the ant column passes always disrupts the traffic, but the ants quickly establish a new trail across the break. If the object is replaced, the ants follow their original trail.

Schneirla (1938:62) reported that the trails of E. hamatum Forel often extend over 400 meters from the bivouac. Ants were observed to resume the use of old trails that had not been used for several days. He stated,

When one considers the heavy rains and the opportunity for volatilization during the intervening days, this ability to utilize the original trail suggest not only the possession of delicately sensitive chemoreceptors by the ants, but also the strength of the qualities which permitted the chemical to persist.

Schneirla and Brown (1950:337) suggested that Eciton chemical trails are re-used more often and can be followed after longer intervals of disuse in dry seasons than in rainy seasons. During the dry period, workers of E. hamatum were observed to follow a trail which they had not used for 31 days. Other

workers of E. hamatum were observed to follow a trail 450 meters long which had not been used for one week. A column of E. burchelli was observed to follow a trail that had been laid by E. hamatum three weeks earlier. Schneirla (1944:3) reported that workers of E. hamatum followed a section of E. burchelli trail to which they had been transported. He stated that this observation suggests a chemical similarity for the trails of closely related army ant species.

Several sources have been suggested for the trail chemicals of various ants. Santschi (1930) suggested that the trail chemical of Tapinoma spp. was from the "anal gland." Carthy (1950, 1951:313) reported that the trail material of Lasius fuliginosus is from the anus. He said formic acid was not the trail chemical. Wilson (1959) presented evidence which indicated the source of the trail chemical of the fire ant, Solenopsis saevissima (Fr. Smith), was the accessory gland of the sting mechanism. No information on the source of the trail chemical of army ants has been reported except for the suggestion by Schneirla (1956:394) that the trail chemical of Eciton hamatum may come from the "anal gland." The term "anal gland" was not defined.

## METHODS

### Collecting and Rearing

During the summer of 1961, five Neivamyrmex colonies were found near Manhattan, Kansas. These included one colony of



carolinensis, two colonies of opacithorax, and two colonies of nigrescens. These colonies were numbered consecutively E-268 to E-272 continuing the numbering series used by Rettenmeyer (1962). These colonies were all found under rocks on grassy slopes. From 2,000 to 10,000 workers were collected from each colony. These workers were collected by aspirating them directly into small plastic bottles and by shoveling workers along with the soil from the nest site into plastic bags. The contents of the plastic bags were dumped into one-gallon, wide-mouth bottles. These bottles were connected to a laboratory nest by a section of plastic tubing, and a 40-watt light bulb was turned on directly over the bottle. In less than 24 hours most of the workers moved into the laboratory nest.

Three types of rectangular frames served as laboratory nests. Sheets of glass were used for tops and bottoms.

Type 1: A large frame was constructed from lumber 1.8 cm. in thickness. The outside dimensions of the frame were 45 cm. by 40 cm. by 3.7 cm. Holes 1.2 cm. in diameter were drilled in the center of the two narrow sides.

Type 2: Small frames were constructed from 1.2 cm. square strips of hard maple. Outside measurements were 22.8 cm. by 22.8 cm. by 1.2 cm. Holes 0.7 cm. in diameter were bored in the center of each of the four sides of the frame.

Type 3: Small frames were molded from plaster of Paris. Outside measurements of the plaster nests were 14 cm. by 15 cm. by 2.5 cm. Walls were 2 cm. thick. One hole 1.6 cm. in diameter was molded into the center of each end of the nest.



Two or three of the above types of nest-boxes, connected by clear plastic tubing, were used to house a sample of army ants. The most successful combination, in which more than 5,000 workers of Neivamyrmex nigrescens were kept alive for over four months, consisted of three small wooden frame nest-boxes connected by plastic tubing. The center nest-box was filled with soil from the original nest site. One of the end nest-boxes was used to provide water, and the nest-box at the opposite end was used as a feeding arena.

Water was supplied by a glass tube extended through a hole in one side of the nest-box. The inner end of the tube was plugged with cotton, and a cork was placed in the outer end. The tube was filled daily with distilled water from a pipette.

Feeding army ants is one of the most difficult problems encountered in attempting to maintain colonies in the laboratory. During this study more than 30 different kinds of prey or food were placed in the laboratory nests. The potential foods tried were: adults and larvae of the confused flour beetle (Tribolium confusum), ants (Camponotus americanus), (Iridomyrmex analis), and (Crematogaster spp.); adults of the following insects: black ground beetle (Harpalus caliginosus), seed-corn beetle (Agonoderus sp.), ground beetle (Galerita sp.), spotted cucumber beetle (Diabrotica undecimpunctata), May beetle (Phyllophaga sp.), cocklebur billbug (Rhodobaenus tredecimpunctatus), differential grasshopper (Melanoplus differentialis), two-striped grasshopper (Melanoplus bivittatus), slant-faced grasshopper (Mermiria sp.), cricket (Gryllus domesticus), brown-banded cockroach (Supella

supellectilium), German cockroach (Blattella germanica), termite (Reticulitermes sp.), lady beetle (Hippodamia convergens), and Collembola (Sinella curviseta), and these foods: sugar, honey, peanut butter, margarine, and hard-boiled egg yolk. Only the cricket (Gryllus domesticus), and the German cockroach (Blattella germanica) were readily eaten.

Between 25 and 50 live German cockroaches or crickets were released into the feeding box each day. The number released depended upon the number of ants in the nest and the number of live cockroaches or crickets remaining in the box from the previous day's feeding.

#### Interspecies Trail Following

Army ants used to determine if one species would follow the trail of another species were Neivamyrmex opacithorax and nigrescens. Nondoryline ant species used were Iridomyrmex analis Andre and Crematogaster laeviuscula Emery.

A trail test arena (Fig. 1) was constructed. Removable panels C and D were placed 0.5 cm. apart in the center of a nest-box of type 2. Number three chromatography paper placed over the glass floor of the arena served as the substrate for the trails. Plastic tubing ("Tygon" formulation B 44-3) 10 cm. in length was inserted into the hole in each side of the arena.

Before each trial, the "trail-arena" was washed with soap and water. The glass was also wiped with 95 percent ethyl alcohol. A square of clean chromatography paper was placed over the floor of the arena. When Iridomyrmex analis or Crematogaster laeviuscula

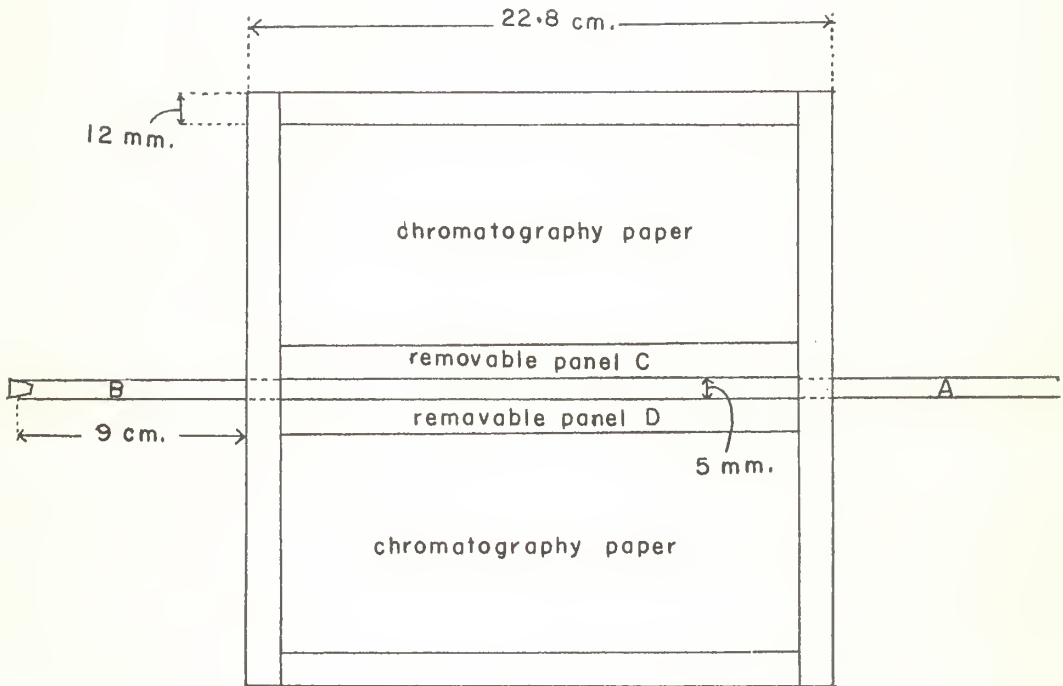


Fig. 1. Top view of trail-arena



trails were needed, it was necessary to place several hundred of their larvae in tube B opposite the nest entrance. Without these larvae, the workers would not readily form a trail through the arena. Approximately one out of five of the above workers would carry larvae back to the nest. Placing larvae in tube B was unnecessary when trails of N. opacithorax or N. nigrescens were being formed. Trails were established by connecting the entrance tube A to a colony nest-box. Normally, in less than five minutes, a column of workers was trailing in both directions across the trail surface.

After the workers had made approximately 1,000 round trips over the trail surface, the entrance tube was disconnected from the nest-box; and the remaining workers were aspirated from the "trail-arena." Panels C and D and tube B were then removed from the arena.

Twenty-five workers of the same or of a different species were released from an aspirator through the hole left by removing tube B. Trail following was rated according to the number of workers that followed the previously established trail to tube A. The ratings according to the number of workers following the trails were as follows: less than 5 workers, "negative" (-); 5 to 10 workers, "weak" (W); 10 to 20 workers, "medium" (M); more than 20 workers, "strong" (S).

N. opacithorax, N. nigrescens, and Iridomyrmex analis were each tested two or more times on different trails of opacithorax, nigrescens, analis, and C. laeviuscula.

### Alcohol Extract Trails

The following methods were used to determine if opacithorax and nigrescens will follow trails of ethyl alcohol extracts of army ants. Extracts of whole workers were obtained by draining alcohol from bottles of workers which had been preserved in 70 percent ethyl alcohol. These extracts had the odor of army ants as a group, and also the odor characteristic of the species. Other extracts were prepared by dissecting the rectums and lower intestines from 20 major workers, and soaking these organs in one milliliter of 70 percent ethyl alcohol for one hour.

Alcohol extracts of whole workers were obtained from samples preserved in alcohol by Carl W. Rettenmeyer on the following dates: N. opacithorax (July 6, 1959), N. nigrescens (May 4, 1957), C. lineolata (March 1, 1959), and Termitopone laevigata (F. Smith) (July 22, 1956). The last species was determined by W. L. Brown. Extracts of intestinal sections were prepared from live opacithorax and nigrescens which had been anesthetized with carbon dioxide. Trails of distilled water, 70 percent ethyl alcohol, and 95 percent ethyl alcohol were used as checks.

Artificial trails were prepared by dipping clean cotton swabs into each liquid and dragging the cotton tips lightly along pencil lines on 15 cm. squares of clean chromatography paper. The cotton tips were dragged over each pencil line three times. These papers were dried in one-gallon, wide-mouth bottles with a layer of "Drierite" (CaSO<sub>4</sub>) 2.5 cm. deep on the bottom. The relative humidity in the drying bottles was less than 5 percent.

Both S-shaped and V-shaped artificial trails were made on the paper. S-shaped trails were 24 cm. long and were used to determine if workers would follow a trail of a particular alcohol extract. V-shaped trails were used to determine if workers preferred alcohol extract trails over distilled water trails. Each "leg" of the "V" trails was 12 cm. long. In preparing "V" trails, one "leg" of the "V" was treated with distilled water and the opposite "leg" of the "V" was treated with an alcohol extract of opacithorax or nigrescens. "S" trails were dried in drying bottles for lengths of time up to seven days. "V" trails were dried for one hour, which was long enough for the ethyl alcohol to evaporate, but not long enough to remove all the water.

The squares of chromatography paper bearing the trails were placed on a flat surface with a single 40-watt bulb directly overhead. Twenty-five workers were released from an aspirator at one end of the "S" trails. A new trail-paper was used for each trial. All parts of the experiment were repeated one or more times with each species. Their trail-following reactions were recorded as indicated under Table 2. Workers were released singly from an aspirator at the junction of the "V" trails. A new trail was used after ten workers were released. Two hundred forty workers of N. nigrescens were released singly at the distilled water-nigrescens alcohol extract junctions of 24 "V" trails. These trails had been dried in drying bottles for one hour to remove the repelling effects of the ethyl alcohol. One hundred sixty workers of N. opacithorax were released singly from an aspirator at the distilled water-opacithorax alcohol extract



junction of 16 "V" trails. These trails had been dried in drying bottles for one hour to remove the ethyl alcohol. After drying, distilled water was again applied to the water "leg" of the "V" trail. The reactions of the workers when released at the junction of the "V" trails were recorded as in Table 3.

#### Source of Odor Trail

This attempt to determine the possible source of the trail chemical or chemicals involved the dissection of various organs from the abdomens of workers of N. nigrescens. Organs dissected were poison gland and vesicle, accessory gland, crop, stomach, and rectum. After the organs were removed, a trail 10 to 20 cm. long was prepared by dragging 6 to 12 of these organs and their contents over sheets of glass or chromatography paper. Trails were dried in drying bottles for lengths of time up to 24 hours. Trails 18 cm. long were also made by dragging the tips of the gasters of whole workers of nigrescens and opacithorax over a glass trail surface. Other trails 15 cm. long were prepared from the extended sting of live workers. To form a trail from the extended sting, a major worker was held between the thumb and forefinger of the left hand. A light squeeze on the abdomen caused the worker to extend the sting and exude a clear fluid. Under a broadfield microscope, a trail was formed by rubbing a clean glass slide in a straight line across the extended tip of the sting. Two of these slides were placed end-to-end to form a trail 15 cm. long.

The trails were tested by releasing workers of N. opacithorax or N. nigrescens from aspirators at the end of the artificial trails. Each part of this experiment was repeated one or more times. Trail following was recorded as positive (+) or negative (-).

An attempt to determine whether the sting was extended or retracted during trail laying was made by viewing the ventral surfaces of workers moving over a clear glass trail surface. The workers were viewed from the ventral surface by using a "Porro" (double right angle) prism and a broadfield microscope.

The pH's of the contents of the stomach, rectum, crop, and poison vesicle were determined by smearing contents from the above organs on "Hydrion" pH indicator paper and comparing the colors of these smears with the "Hydrion" color chart.

## RESULTS AND DISCUSSION

### Interspecies Trail Following

The results of testing Neivamyrmex opacithorax, N. nigrescens, and Iridomyrmex analis on trails made by live opacithorax, nigrescens, analis, and Crematogaster laeviuscula are summarized in Table 1. Both species of army ants followed their own trails and the trails of the other species of army ants, but would not follow trails laid by Iridomyrmex or Crematogaster. Iridomyrmex analis followed its own trail, but did not follow trails laid by the army ants, or Crematogaster. The workers of opacithorax appeared to follow the trails of nigrescens better than nigrescens

Table 1. Interspecies trail tests.

Species tested on trails	Trails tested			
	<u>N.</u> <u>opacithorax</u>	<u>N.</u> <u>nigrescens</u>	<u>I.</u> <u>analís</u>	<u>C.</u> <u>laeviuscula</u>
<u>N. opacithorax</u>	S	S	-	-
<u>N. nigrescens</u>	M	S	-	-
<u>I. analís</u>	-	-	S	-

Number of workers following trails: (-) = less than 5;  
(M) = 10 to 20; (s) = 20 or more.

followed the trails of opacithorax. Further work will have to be done to determine whether this difference is significant and whether it may be related to the larger size and faster speed of nigrescens. Both species of army ants were able to follow each other's trails after the paper had been in drying bottles 48 hours. Army ants released at the edge of the arena where no trail was present collected in a small group and then began running slowly over the surface in one or two columns which often followed the walls of the arena. In contrast, when a trail was present, the ants without hesitating quickly followed the trail across the arena.

#### Alcohol Extract Trails

Results using trails of alcohol extracts are summarized in Table 2. Each part of this experiment was repeated one or more times with each species. Workers of opacithorax and nigrescens were attracted to distilled water and repelled by 95 percent ethyl alcohol. Workers readily followed trails of distilled water.



Table 2. Responses of nigrescens and opacithorax to trails of alcohol extracts of whole workers.

Trail materials	Periods of drying				
	: Wet	:15 min.:	4 hrs.	:10 hrs.:	7 days
Distilled water	+	+	-	-	-
Ethyl alcohol (70%)	-	+	-	-	-
Ethyl alcohol (95%)	-	-	-	-	-
<u>N. opacithorax</u> extracts	-	+	S	S	+
<u>N. nigrescens</u> extracts	-	+	S	S	+
<u>C. lineolata</u> extracts	-	+	-	-	-
<u>T. laevigata</u> extracts	-	+	-	-	-

Number of ants following trails: (-) = less than 5;  
(+) = 5 or more; (S) = 20 or more.

They also followed trails of 70 percent ethyl alcohol after the alcohol had been allowed to evaporate for 15 minutes. Since ants were repelled by 95 percent ethyl alcohol and unevaporated 70 percent ethyl alcohol, the trail-following response to the 70 percent ethyl alcohol (evaporated 15 minutes) is probably to the remaining water and not to the alcohol. Trails of distilled water and trails of 70 percent ethyl alcohol could not be followed after four hours in drying bottles. Trails prepared from alcohol extracts of whole workers of opacithorax and nigrescens were still followed after seven days in drying bottles. Alcohol extract trails of Crematogaster lineolata and Termitopone laevigata were not followed after four hours in drying bottles.

After evaporation of the alcohol, the alcohol extract trails of opacithorax and nigrescens exuded a strong "fecal" odor very

similar to the odor of the trails prepared by smearing freshly dissected rectums on chromatography paper.

The ethyl alcohol extract trails prepared from the lower intestines and rectums of nigrescens and of opacithorax were each followed by both nigrescens and opacithorax. These trails were still followed readily after drying 108 hours in drying bottles.

Since workers of both species studied readily followed trails of distilled water, "V" type trails were employed to determine if there was a preference for the alcohol extract trails over the distilled water trails. Results are presented in Table 3. Out of 240 single releases, 228 workers of nigrescens (95 percent) chose the alcohol extract trails of nigrescens and 12 workers chose the distilled water trail. Out of 160 single releases, 143 workers of opacithorax (89 percent) chose the alcohol extract trails of opacithorax and 17 workers chose the distilled water trails. The above results indicate both species follow trails prepared from ethyl alcohol extracts of whole workers much more readily than they follow trails of distilled water. Except for the odor perceptible to a person and the fact that the army ants readily follow both trails, there is no evidence that the alcohol extracts contain the same material as the rectum. After four years at room temperature, the alcohol extracts still contain some chemical(s) which elicit trail following.

Table 3. "V" trail experiments with N. nigrescens and N. opacithorax.

"V" trails No.	Number of <u>nigrescens</u> workers following trails		Number of <u>opacithorax</u> workers following trails	
	Distilled	<u>nigrescens</u>	Distilled	<u>opacithorax</u>
	water	extract	water	extract
1	1	9	0	10
2	2	8	0	10
3	0	10	0	10
4	0	10	2	8
5	0	10	0	10
6	0	10	0	10
7	1	9	2	8
8	2	8	3	7
9	1	9	1	9
10	0	10	0	10
11	1	9	3	7
12	0	10	3	7
13	0	10	2	8
14	2	8	1	9
15	0	10	0	10
16	0	10	0	10
17	0	10		
18	1	9		
19	0	10		
20	0	10		
21	0	10		
22	0	10		
23	1	9		
24	0	10		
Totals	12	228	17	143

#### Source of Odor Trail

By viewing the ventral surface of workers of N. nigrescens moving over a clear glass trail surface, the workers' stings were seen to be fully retracted during the process of trail laying. After several workers had traveled over the glass surface, small droplets could sometimes be seen by viewing the surface with a compound microscope. The origin and significance of these



droplets were not determined. Workers of nigrescens and opacithorax readily followed their own trails prepared by dragging the tip of the gaster of live workers over a glass trail surface, but neither species followed trails prepared by dragging glass slides over the extended stings. Workers of both nigrescens and opacithorax readily followed trails made from the contents of the rectum and from contents of the stomach of nigrescens, but did not follow trails made from the contents of the crop, the poison vesicle, or the accessory gland. The trails of stomach and rectal contents were still followed strongly after 24 hours in drying bottles. Using "Hydrion" pH indicator paper, the pH's of the contents of the stomach and rectum were found to be approximately five; and the pH's of the contents of the crop and poison vesicle were approximately seven. The odor of the trails made from rectal contents seemed to the author to be almost identical with the odor of the dried trails prepared from alcohol extracts of whole workers. The above data suggest that the contents of the rectum are used to make the chemical trail.

#### SUMMARY

Trail-following by army ants of the genus Neivamyrmex was investigated in the insect behavior laboratory at Kansas State University during the summer of 1961. Samples of Neivamyrmex carolinensis, N. opacithorax, and N. nigrescens were collected within a five-mile radius of Manhattan, Kansas. The investigations were divided into three areas: interspecies trail following, artificial trails made from alcohol extracts, and source of

chemical trails.

Each species of Neivamyrmex could follow trails of the other army ant species, but neither species of army ants followed trails of I. analis or C. laeviuscula. These results add further weight to the suggestion by Schneirla (1944) that army ants of one species can follow the trails of other closely related species.

Workers of opacithorax and nigrescens followed trails made from ethyl alcohol extracts of whole workers and of rectal contents of opacithorax and nigrescens, but neither army ant species followed trails made from ethyl alcohol extracts of C. lineolata or T. laevigata. Since the above trails were still followed after seven days in drying bottles, it was concluded that drying has little effect upon the usable life of these artificial trails.

The apparent source of the trail chemical or chemicals of nigrescens is the stomach or lower intestines. The pH of the contents of these organs is approximately five. It was not determined whether the trail substance is the fecal material itself or a special secretion added to the fecal material.

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LABORATORY EXPERIMENTS OF THE TRAIL FOLLOWING  
OF ARMY ANTS OF THE GENUS NEIVAMYRMEX  
(FORMICIDAE: DORYLINAE)

by

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Trail following by army ants of the genus Neivamyrmex was investigated in the insect behavior laboratory at Kansas State University during the summer of 1961. Workers from colonies of Neivamyrmex carolinensis (Emery), N. opacithorax (Emery), and N. nigrescens (Cresson) were collected near Manhattan, Kansas. Workers of nigrescens lived for over three months in laboratory nests. The laboratory investigations were divided into three areas: interspecies trail following, artificial trails made from ethyl alcohol extracts, and source of odor trails.

The purpose of the interspecies trail following experiments was to determine if one species of Neivamyrmex can follow the chemical trails of another. Trails of Crematogaster laeviscula Emery, and Iridomyrmex analis Andre were also tested to determine if the army ants could follow trails of ants other than army ants. Trails were prepared by allowing workers of one species to form a column between removable panels of a rectangular nest-box. After approximately 1,000 round trips over the paper surface of the nest-box, the original workers and trail panels were removed, and 25 workers of the same or different species were released at the end of the established trail. The army ants readily followed their own and each other's trails but did not follow trails of C. laeviuscula or I. analis.

Extracts of whole workers were obtained by draining the fluid from bottles of workers which had been preserved in 70 percent ethyl alcohol two to four years previously. Additional extracts were prepared by dissecting the rectums and part of the intestines from 20 major workers and soaking these organs in 1 ml.



of 70 percent ethyl alcohol for one hour. Distilled water, 70 percent ethyl alcohol, 95 percent ethyl alcohol, and ethyl alcohol extracts of Crematogaster lineolata and Termitopone laevigata were used as controls. Trails were prepared from the above materials by dragging the tips of cotton swabs over chromatography paper. These papers were dried for periods up to seven days in drying bottles containing a 2.5 cm. layer of "Drierite" ( $\text{CaSO}_4$ ) in a tightly capped one-gallon wide-mouth bottle. Trails were tested by releasing 25 workers from an aspirator at the end of the extract trails. Workers of opacithorax and nigrescens followed trails of distilled water and trails of 70 percent ethyl alcohol after the alcohol had evaporated for 15 minutes, but did not follow either of the above trails after they had dried for four hours in drying bottles. Both species of army ants readily followed trails prepared from alcohol extracts of either army ant after the trails were dried for periods up to seven days, but neither species of army ant followed alcohol extract trails of C. lineolata, or T. laevigata. Both species of army ants followed trails of alcohol extracts of rectums of either species after periods in drying bottles up to 108 hours.

The following possible sources of the chemical trails of Neivamyrmex were investigated: poison glands, accessory glands, crops, stomachs, and rectums. Trails were prepared by dragging six to twelve of each of the above organs dissected from major workers of nigrescens over chromatography paper surfaces. Trails were tested by releasing workers of nigrescens or opacithorax

from an aspirator at the end of the trail. Both opacithorax and nigrescens followed trails prepared from rectums and from stomachs of nigrescens, but did not follow trails prepared from crops, poison glands, or accessory glands. Thus, the army ants probably make their trails by depositing minute amounts of the rectal contents.